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# Synthesis and Characterization of Cuo Nanoparticles Stabilized by Quercetin and Its Application for Anti-Breast Cancer Activity

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**Abstract**

The main idea in this research used quercetin (QT) as a stabilizing agent to prepare CuO nanoparticle (NPs). QT is a pigment which exists from plants, fruits, and in some medicines. It contains numerous hydroxyl groups, works as a complexing agent and a self-assembly agent. The prepared CuO-NPs were characterized by Fourier transformed infrared (FT-IR), X-ray diffraction (XRD), scanning and transmission electron microscopy (SEM and TEM), and energy-dispersive X-ray analysis (EDX). The structure investigation confirm that the CuO-NPs were polycrystalline nature having a monoclinic crystalline form and showed (11$\bar{1}$) and (111) preferential orientations. The estimated particle sizes by XRD, TEM, and SEM ranged from 12.03 to 15.3 nm. Surface analysis displayed that homogeneously distributed CuO-NPs with a spherical shape. On the other hand, synthesized CuO-NPs were tested for anticancer activity on the human breast. The infected cell (MCF-7) uses MTT (3-(4,5-dimethyl-2-triazolyl)-2,5-diphenyl-2-tetrazolium bromide) test. The inferred CuO-NPs clarify high anticancer cytotoxicity of the (MCF-7) with an IC50 μM value of 57.6 and 42.8 μg/mL for 24 and 72 h respectively.

***Keywords***: Synthesis; CuO Nanoparticles; Quercetin; Anticancer; Breast cancer activity.

## Introduction:

In recent years, Nanoparticles are being employed in a wide range of applications using scale from bulk to nanometre size [1]. Human cells (around 7μm) are significantly bigger than the nanoparticles [2]. Nowadays, transition metal- nanoparticles have attracted the attention of researchers due to their capacity to exert cytotoxicity on various cells through oxidative stress [3, 4]. Among various metal transition nanoparticles, copper oxide nanoparticles (CuO-NPs) and their derivatives have attracted the interest of researchers due to their rapid synthesized, high stability; furthermore, it gives a range of potentially important physical properties compared to other metal nanoparticles [5-6] Breast cancer disease is a standout amongst the most hazardous illnesses faced by ladies in advanced countries [7-8] . Precipitation [9] CuO-NPs have numerous applications particularly in the biological and medical field, which includes the bio-control agent [10], anti-oxidation properties [11], efficient

anti-micro bacterial agent [12], drug delivery [13], and anticancer activity [14].

Breast cancer is a great danger to cells (MCF-7), and is leading to death in women [15-16]. Currently, breast cancer

( MCF-7 is the acronym of Michigan Cancer Foundation-7, referring to the institute in Detroit where the cell line by Herbert Soule and co-workers) cells are primarily treated *via* surgical resection and numerous adjuvant chemotherapy drugs utilized as a part of the treatment protocol particularly in instances of early finding [17-18]. Evaluate the site-specific drug delivery of 5-FU with chitosan (CS) as a carrier and quercetin (Qu) against induced colon cancer in Wistar rats [19]. However, modern medical research in effective cancer therapy faces many challenges like the mechanisms, tumor, and the restrictions related to utilized cancer therapeutic options [20]. Recently, several researchers focus their efforts on the nanoparticles as a promising carrier system for the delivery of chemotherapeutic agents by using both active and passive targeting to avoid systemic toxicity or normal cell toxicity. Many parameters affect cell toxicity depending on the unusual properties of nanoparticles (e.g. size, shape, aggregation, solubility, and optical features), the exposure time, and concentration of nanoparticles [21-23]. Numerous natural compounds have been proved to have the possibility to inhibit cancer cells at cellular and animal levels, like ligands, flavonoids [24-26]. Preparation of compounds by green chemistry large annular complexes [27-28]. Quercetin (QT) is a plant pigment flavone of the polyphenols, it exists in many fruits, contain a large amount of QT [29-30]. More studies have indicated that QT has high antioxidant, anti-allergic, anti-inflammatory, antiviral, and therapeutic effects against various types of cancer [31-32].

The aim of this work was to synthesize the CuO-NPs using QT as a reducing and capping agent. CuO-NPs were

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characterized using numerous methods such as FT-IR, XRD, TEM, and SEM. The anti-breast (MCF-7) cancer activity was studied by the QT-capped CuO-NPs, where the QT is working as an enhancing agent with the CuO-NPs.

## Experimental

### Materials

The following analytical grade materials were used without any further purification: copper sulphate pentahydrate ($CuSO_4.5H_2O$), quercetin ($C_{15}H_{10}O_7$), and (NaOH) were purchased from Sigma-Aldrich. All analytical grade reagents were prepared with deionized water (D.W.). For the cytotoxicity test, cell culture media and reagents including fetal bovine serum (FBS), Dulbecco's modified eagle's medium (DMEM), dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich.

### Synthesis of CuO-NPs using QT

For synthesis of CuO-NPs, 0.1 M of $CuSO_4.5H_2O$ solution was prepared by dissolving 2.496 g of $CuSO_4.5H_2O$ in D.W., the volume completed to 250 mL in volumetric flask. $CuSO_4.5H_2O$ solution was mixed with 30 mL of QT (3.38 g of QT dissolved in 50 mL deionized water). The reaction mixture was stirred 10 min, and then 0.1M NaOH was added slowly until the pH arrived at About 10. The mixture was stirred for 2 hours at 70 $^{O}$C. At the end of the reaction, it got a brown-black colored precipitate. The precipitate was separated by filter funnels 11G (capacity 50 mL and washed with D.W. several times and dry at 60°C for 3 hours and the oven was used for diagnosis. Scheme 1 shows the illustration route of the synthesis of CuO-NPs [33].

### Cell culture and cell viability evaluation

The cell lines MCF-7 (human breast adreno carcinoma) was grown in Dulbecco's modified Eagle medium (DMEM) cell culture medium containing 10% fetal bovine serum, 100 U/mL penicillin and streptomycin (100 mg $mL^{-1}$) under a humidified atmosphere with 5% $CO_2$ at 37 °C. in T-75 flasks and were subcultured twice a week. For the assays, cells (2000 cells/well in 200 µL of complete DMEM) were seeded in each well of ULA 96-well round-bottom plates and to encourage cell aggregation. MCF-7 cells were then exposed for 24 and 72 h in DMEM supplemented with varying concentrations (10, 20, 30, 50 and 100 µg/mL) of CuO-NPs.

### Checking account poisons analysis

All the tests are thorough as the mean ± SD of three replicates. p values < 0.05 was considered as statistically significant. Origin Lab software 2018 (9.5) was used for all statistical analysis.

### Characterization of CuO-NPs

The purity of phase and crystalline size of synthesized CuO-NPs with quercetin were characterized by X-ray diffraction using a (PAN-analytical), with Cu K-alpha wavelength radiation (λ= 1.5406 Å). The crystalline phase was identified using the Joint Committee on Powder Diffraction Standards (JCPDS) database. Functional groups and chemical composition of copper oxide nano powder (as pellets in KBr) were analyzed by FT-IR Shimadzu 8400 spectrophotometer over the range of (400 - 4000 $cm^{-1}$). The surface morphology of the CuO-NPs was inspected by field emission scanning electron microscopy (FE-SEM) (Model: TESCAN MIRA3) with energy dispersive X-ray analysis (EDAX) was done using the same instrument and transmission electron microscopy (TEM) (Model: ZEISS-EM10C) at operating voltage 100 Kv.

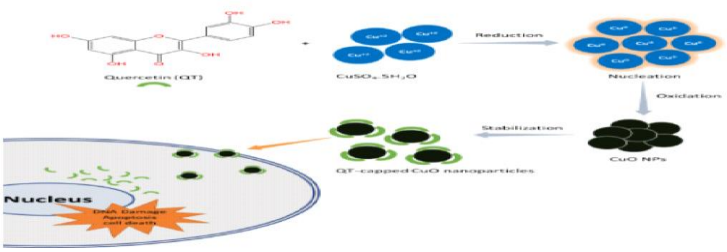
## Results and Discussion

### Characterizations of CuO-NPs

### XRD and FT-IR spectra analysis

The Figure 1 (A) depicts the XRD pattern of CuO-NPs which is synthesized by assistance of QT. It can be seen that CuO powder possesses sharp characteristic diffraction peaks of (110), (11$\bar{1}$), (111), (20$\bar{2}$), (020), (202), (11$\bar{3}$), (31$\bar{1}$), (220), (311), and (004) crystal planes which appeared at 2Θ values of 32.6°, 35.5°, 38.5°, 48.8°, 53.4°,58.1°, 61.5°, 66.0°, 68.0°, 72.3° and 74.8° respectively which can be attributable to monoclinic phase (space group $C_2/c$) of CuO (JCPDS number: 00- 048-1548) without any peaks belonging to other copper oxide phase ($Cu_2O$, $Cu_4O_3$, etc.). The average crystallite size of the CuO-NPs (D) was obtained using the Debye-Scherer equation [35] which was about 12.03 nm. The presence of sharp peaks and crystallite sizes smaller than 100 nm indicates the Nano crystalline nature of CuO-NPs of CuO-NPs [3].

But Figure 1. (B) showed the FT-IR spectra, recorded in order to characterize the surface structure and functional groups involved in the CuO-NPs of FT-IR analysis for CuO-NPs, shows a strong and broad absorbed at 3340 $cm^{-1}$ (OH ), the shoulder peak at 1708 $cm^{-1}$ assigned C=O group, the weak band at 1026 $cm^{-1}$ can be assigned to the C-O-H stretching vibrations of QT and bands at (514 , 424) $cm^{-1}$ modes of bending vibration of the Cu–O bond .




Scheme 1. (illustration route of the synthesis of CuO-NPs) using QT and overview of the most probable intracellular mechanism of action of the QT-capped CuO-NPs [34].

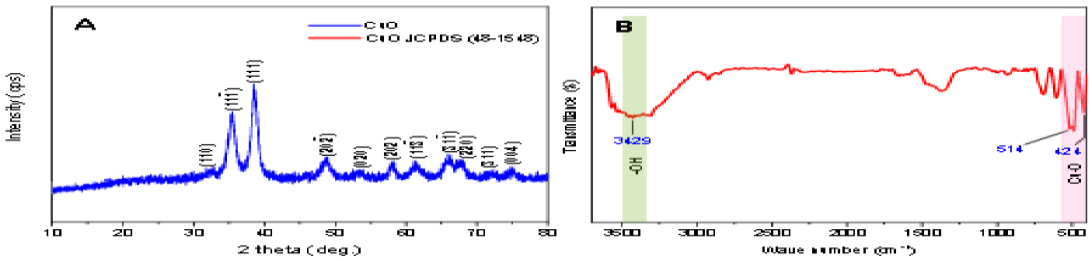



Figure 1. (A) XRD patterns of CuO-NPs; (B) FT-IR spectrum of CuO-NPs.

**Morphology measurements**

The morphology and surface structure of the CuO-NPs were studied through SEM/EDX and TEM. FESEM images at different magnifications showed QT-capped CuO-NPs which had spherical shapes with a regular and smooth surface. Figure 2. (A and B) showed the particles were small with minimum agglomeration. As shown in Figure 2. (C) EDX was used to investigate the composition of the synthesized CuO-NPs, where the atomic ratio of the copper signal was about 31.68% .

Additionally, the EDX profile observed a presence of C and O peaks. This indicates the presence of QT which leads to the appearance of large amounts of C in this analysis. Thus, this indicates it could be connected with CuO-NPs. The TEM image (Figure 2. (D)) showed the formation of crystalline phase in the CuO-NPs, which have a spherical shape free from agglomeration and are well dispersed with an average diameter of 14.78 ± 0.5 nm with a range of 7.5-27.5 nm (Figure 2. (E)). The value of the average diameter is in good agreement with the 12.03 nm value obtained from XRD pattern using Scherer's equation.

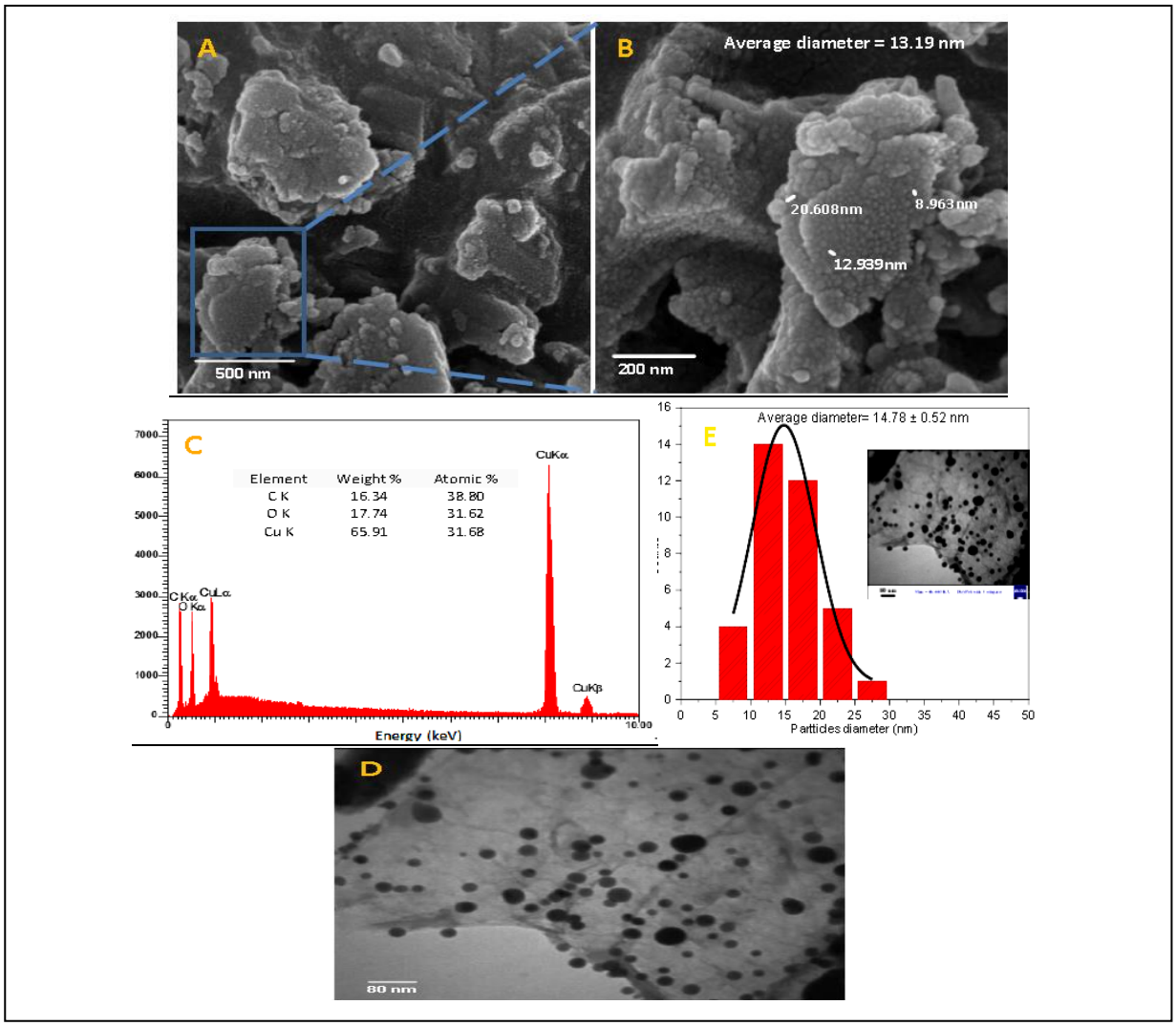



**Figure 2.** (**A** and **B**) FE-SEM images of CuO-NPs at different magnifications, (**C**) EDX spectrum of CuO-NPs. (Inset; Quantitative elemental analysis), (**D**) TEM for CuO-NPs, (**E**) particle size distribution histogram.

## Cytotoxicity assay

In *vitro* anticancer activity of the synthesized QT-capped CuO-NPs was evaluated against a panel of human cancer cell lines such as breast (MCF-7), and a normal human dermal fibroblast (NHDF) cell line using MTT assay. It is a sensitive colorimetric assay for the determination of the number of survivable cells in cell proliferation and cytotoxicity assays, based on the mitochondrial reduction of the tetrazolium salt by actively growing cells to produce blue water-insoluble formazan crystals. It is well known that CuO can produce strong oxidant OH• as reactive oxygen species (ROS) [36], biomedical and clinical sectors [37] and radical scavenging [38]. In this study, it capped CuO-NPs by QT in order to enhance the efficiency of QT delivery, thus treating cancer cells. Therefore, different concentrations of QT-capped CuO-NPs were incubated with MCF-7 cells. The concentration-dependent effect for QT capped CuO-NPs on MCF-7 cell survivability was analyzed. Figure 3 exhibits the proliferation rate, related to non-exposed control cells, evaluated after 24 and 72 h exposure to QT capped CuO-NPs at different concentrations extending from (10, 20 30, 50, and 100 µg/mL). From Figure 4 (A, B, C), it can be seen also that the cells significantly unaffected in 24 h after treatment with 100 µg/mL of QT capped CuO-NPs. However, after 72 h of treatment, cell survivability tends to significantly decrease (38.7%) in MCF- 7 cells, where ~ 61% of the cell died. It is because the surface marker (QT receptor) is on target cells [39]. Additionally, normal cells have the ability to tolerate a certain amount of exogenous ROS stress, due to their antioxidant capacity [40]. The suggested mechanism of cell death appears through apoptosis as shown in Scheme 1. On the other hand, QT capped CuO-NPs showed potent activity against MCF-7 cells line at IC50 values 57.6 and 42.8 µg/mL for 24 and 72 h respectively.

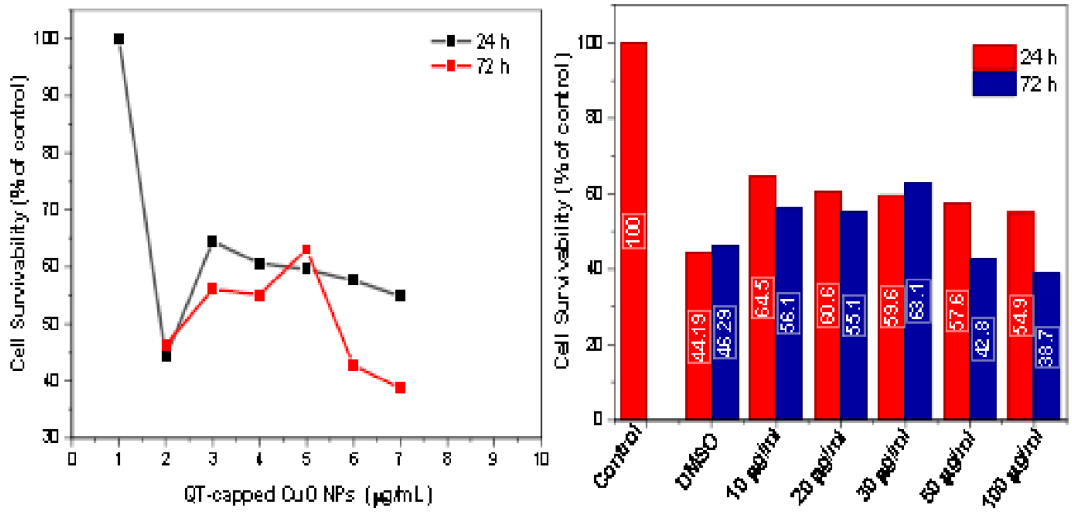



Figure 3. Effect of 100 µg/mL of QT capped CuO-NPs on the cell survivability of MCF-7 at 24 and 72 h .by MTT assay

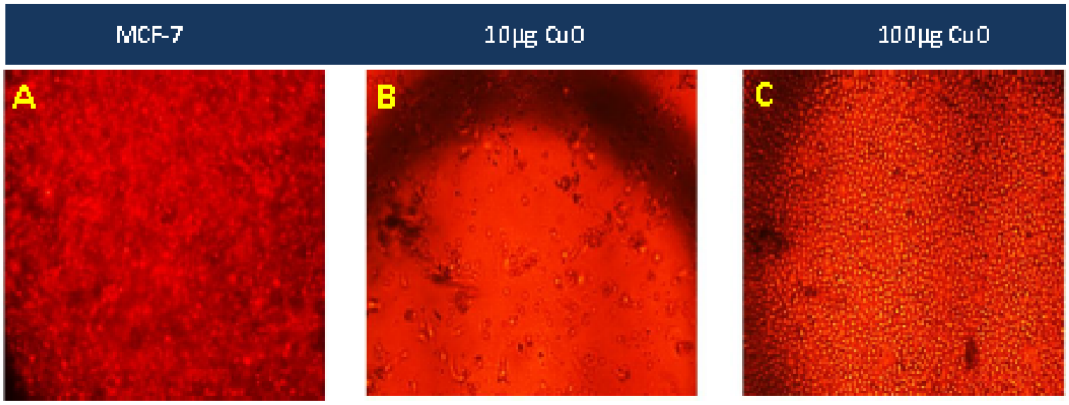



Figure 4. The potent activity against cancer cell lines with QT capped CuO-NPs for 72 h (A) normal cells, (B) 10 µg/mL, (C) 100 µg/mL

## Conclusion

In this research, we succeed at synthesizing QT-capped CuO-NPs. These nanoparticles exhibited a spherical morphology as SEM and TEM results shows. FT-IR spectra confirmed the presence of Cu–O bonding showed XRD paradigm revealed the phase of CuO NPs. Generally, in *vitro*, the results refer to the effectiveness of our synthesized QT-capped CuO-NPs for treatment breast cancer. Over and above, all the synthesized nanoparticles offer considerable higher effected on breast cancer cell lines without any effect against the normal cells. QT-capped CuO NPs concentration 100 mg/mL showed large effect on anti - cell lines and the propose mechanism of cell death appears through apoptosis. The strong efficacy of QT-capped CuO NPs concentration 100 mg/mL may be due to the strong effect of additional phytochemicals present in the Quercetin used to synthesize this nanoparticle. The results we obtained compared to the published research. Our results were the best because it is environmentally friendly and effective against breast cancer well.

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# تحضير وتشخيص أوكسيد النحاس النانوي المستقرة بواسطة كيرسيتين وتطبيقاته مضاد لسرطان الثدي

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**الخلاصة**

الفكرة الرئيسية لهذا البحث هو استخدام كيرسيتين (QT) كعامل استقرار لتحضير أوكسيد النحاس النانوية هي صبغات موجودة في النباتات والفواكه وفي بعض الأدوية. QT تحتوي على العديد من مجموعات الهيدروكسيل، تعمل كعامل ماسك وعامل تجميع ذاتي، وقد شخصت الجسيمات النانوية لأوكسيد النحاس بطيف فوريه الأشعة تحت الحمراء(FT-IR) ، وحيود الأشعة السينية(XRD) ، (والميكروسكوب الإلكتروني الماسح SEM والمجهر الالكتروني النافذ TEM)، وتحليل بمطياف تشتت الطاقة بالأشعة السينية(EDX). واكد التشخيص التركيبي بأن الجسيمات النانويةCuO كانت ذات طبيعة متعددة البلورات ولها شكل بلوري أحادي الميل وأظهرت (111) و(111) لتميز متوفقة بأنه أحجام الجسيمات المقدرة بواسطة XRD و TEM و SEM تراوحت ( 12.03 - 15.3) نانومتر. أظهر تحليل السطح أن أوكسيد النحاس النانوية الموزعة بشكل متجانس ذات شكل كروي. ومن ناحية أخرى، أوكسيد النحاس النانوي المحضر قد اختبر كمضاد للسرطان الثدي البشري وفعاليته. تستخدم الخلية المصابة (7-MCF) مؤسسة ميشيغان للسرطان واختبار MTT (3-(4,5-dimethyl-2-triazolyl)-2,5-diphenyl-2-tetrazolium (bromide. استنتج بأن أوكسيد النحاس النانوية المحضرة أظهرت سمية عالية كمضادة للسرطان لـ (7-MCF) بقيمة 50IC ميكرومتر 57.6 و42.8 ميكروغرام/ مل لمدة 24 و72 ساعة على التوالي.